9-20-2017

Development and large scale manufacturing of exosome-based therapeutics

Konstantin Konstantinov
Codiak Biosciences, USA

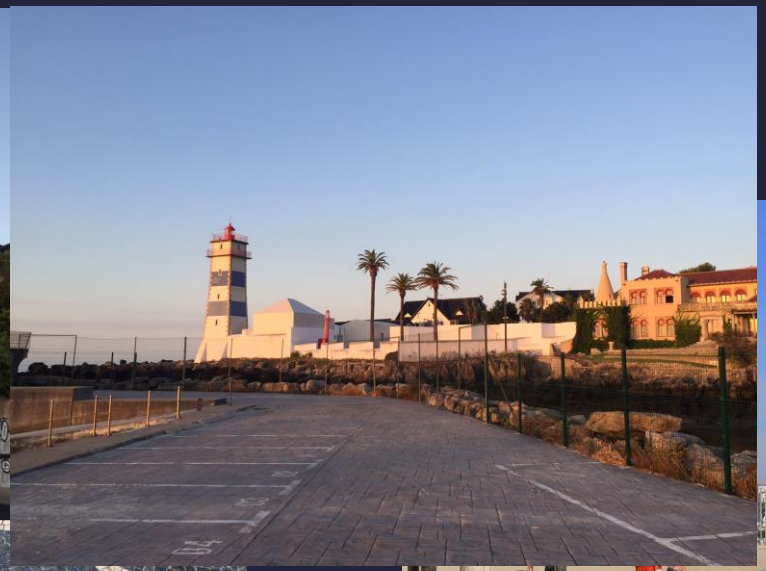
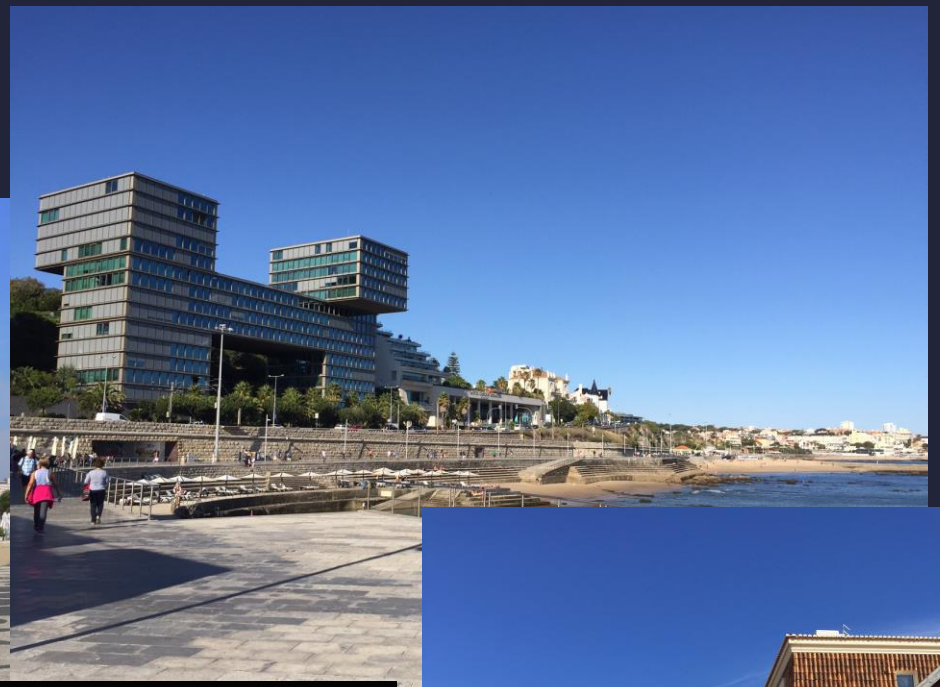
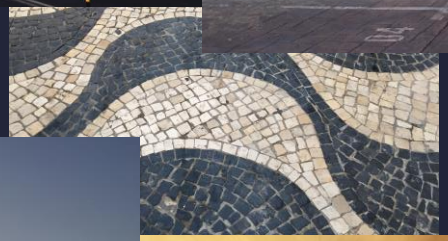
Development and Large Scale Manufacturing of Exosome-Based Therapeutics

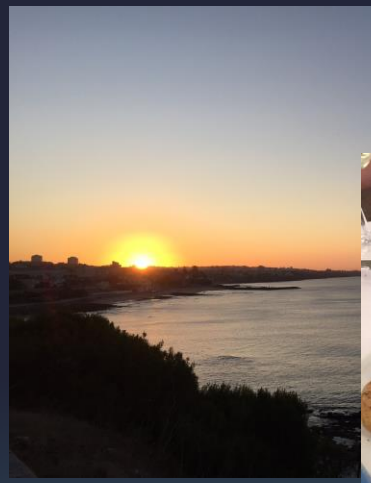
Konstantin Konstantinov, Ph.D.
SVP, Manufacturing & Process Sciences

Integrated Continuous Biomanufacturing III Conference
Hotel Cascais Miragem, Cascais, Portugal, Sep 17-21, 2017

CODIAK

Beautiful Cascais

Brief history of my involvement with continuous bioprocessing

1970s: SCP continuous process using methanol (ICI)

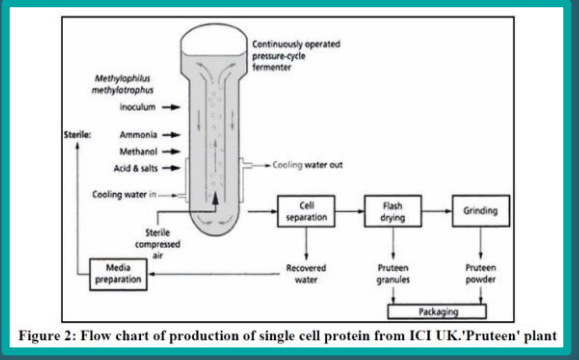


Figure 2: Flow chart of production of single cell protein from ICI UK.'Pruteen' plant

1989: B&B - SCP continuous process optimization & control (Osaka U)

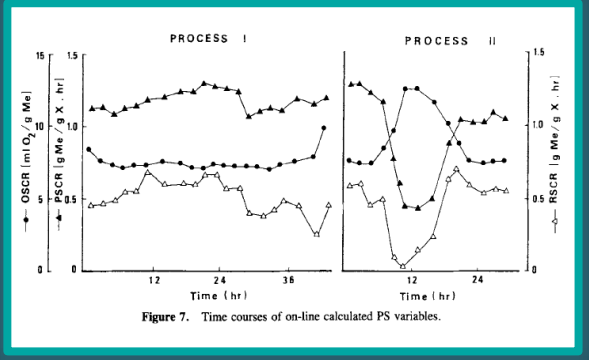
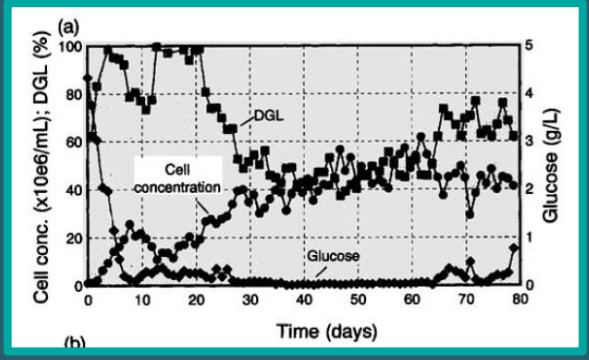


Figure 7. Time courses of on-line calculated PS variables.

1996: B&B – large scale continuous NS0 process for Mab (Bayer)



2002: B&B – USP/DSP integration using annular chrom (Bayer)

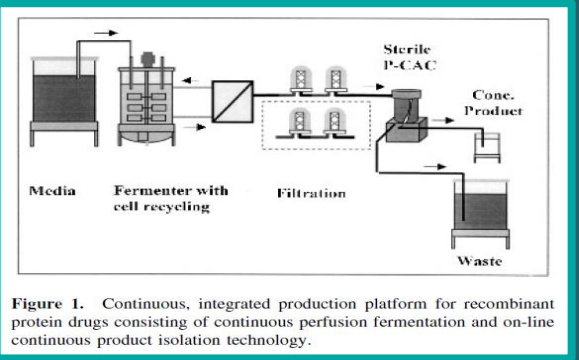
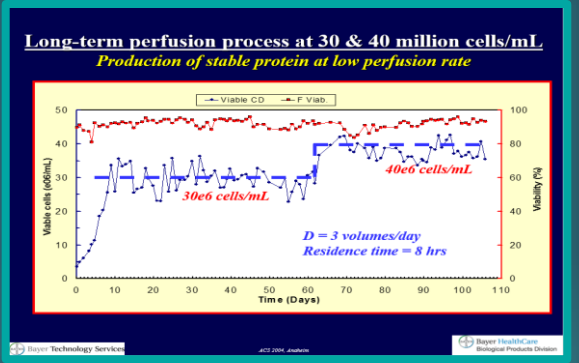
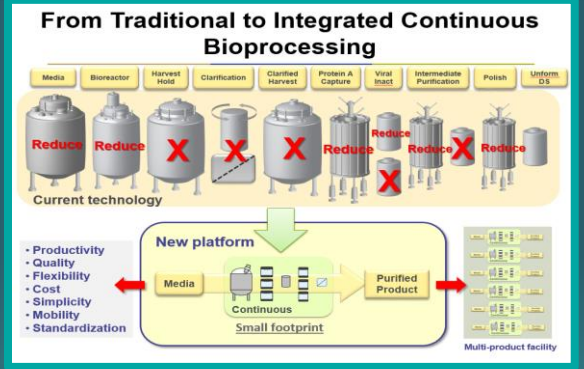


Figure 1. Continuous, integrated production platform for recombinant protein drugs consisting of continuous perfusion fermentation and on-line continuous product isolation technology.

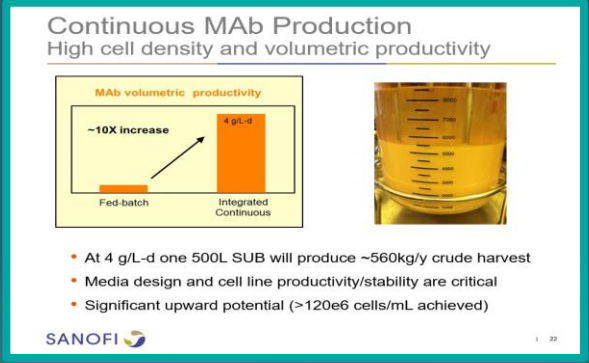
2004: ACS industrial award to Bayer for perfusion technology dev



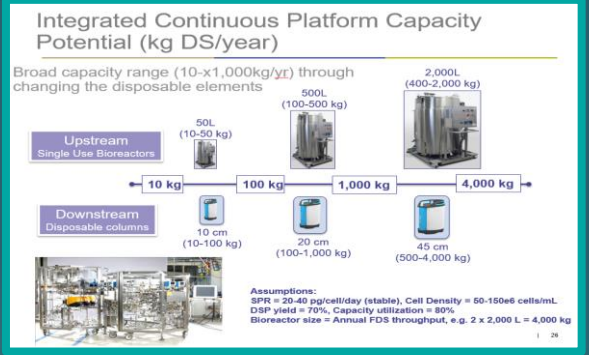
2012: B&B – Integrated continuous biomanufacturing (Genzyme)



2014: Progress with continuous Mab production (Genzyme)



2015: Capacity of a fully integrated continuous system (Genzyme)



The principle of requisite variety

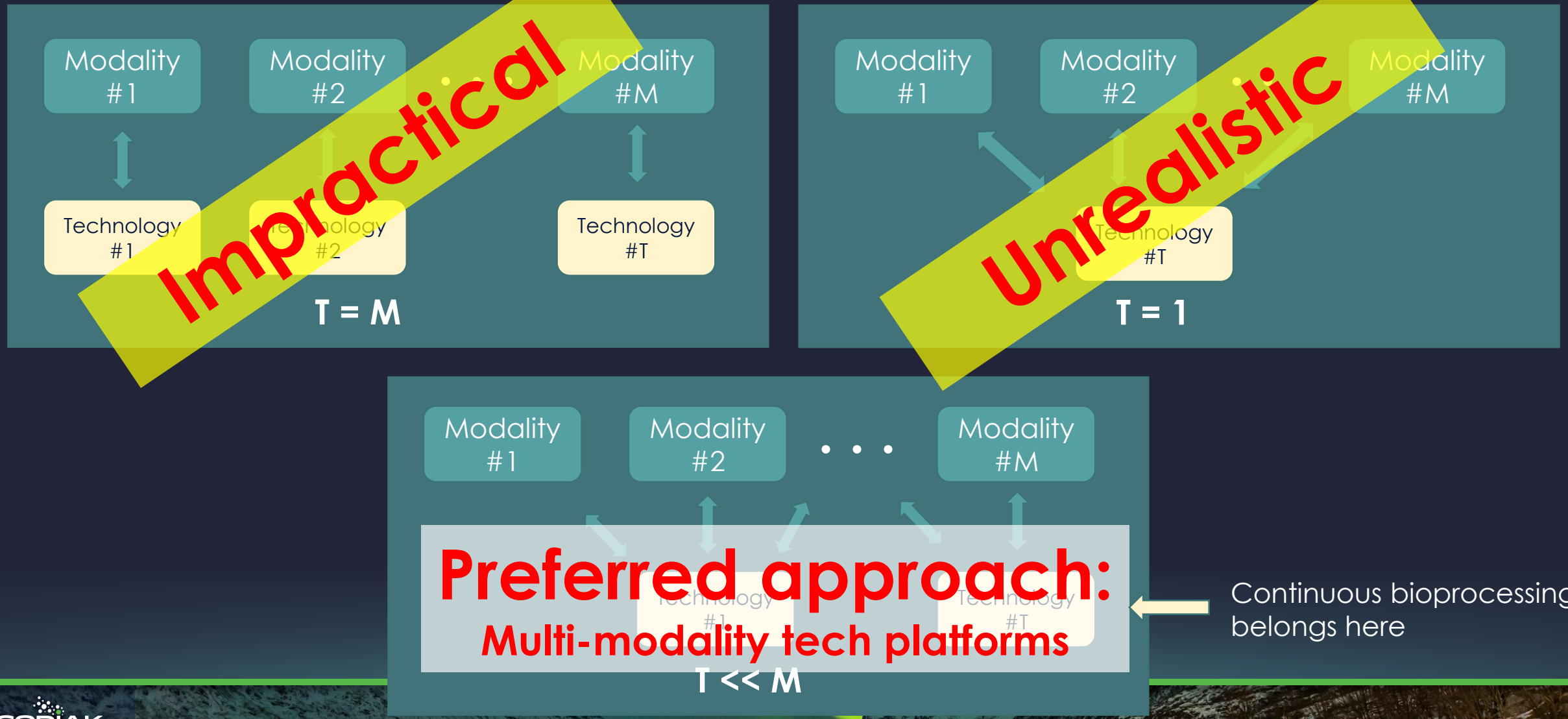
W. Ross Ashby (1956): An Introduction to Cybernetics

If a system is to be stable, the number of states of its control mechanism must be greater than or equal to the number of states in the system being controlled.

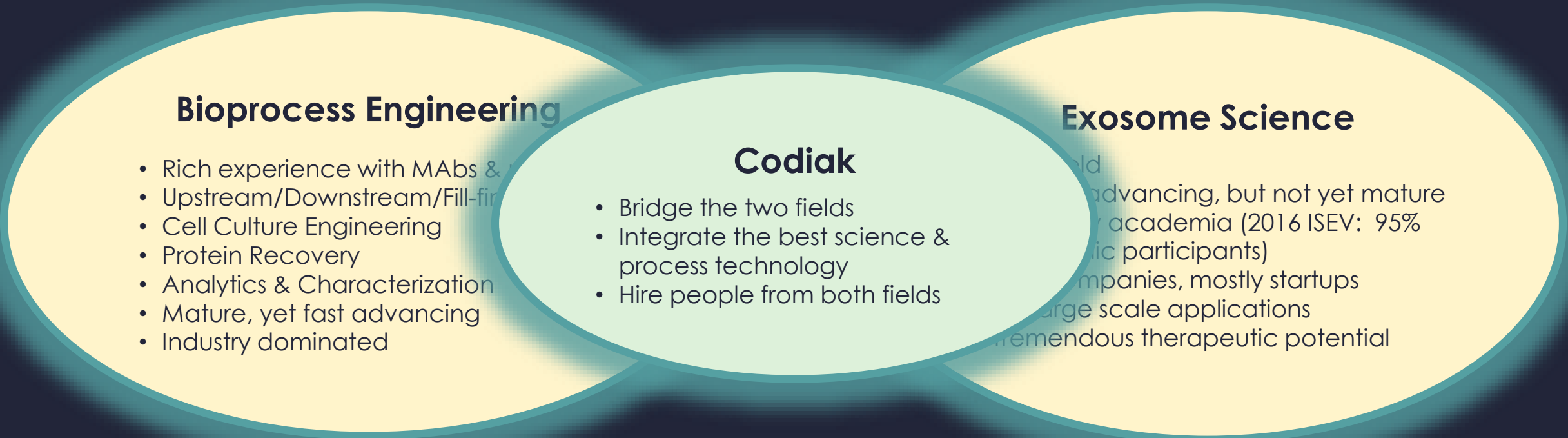
" …. only variety can destroy variety "

Exosomes: A new modality with a tremendous therapeutic potential

Biotherapeutic Modalities and Technologies for Their Manufacturing: Importance of Multi-Modality Production Platforms

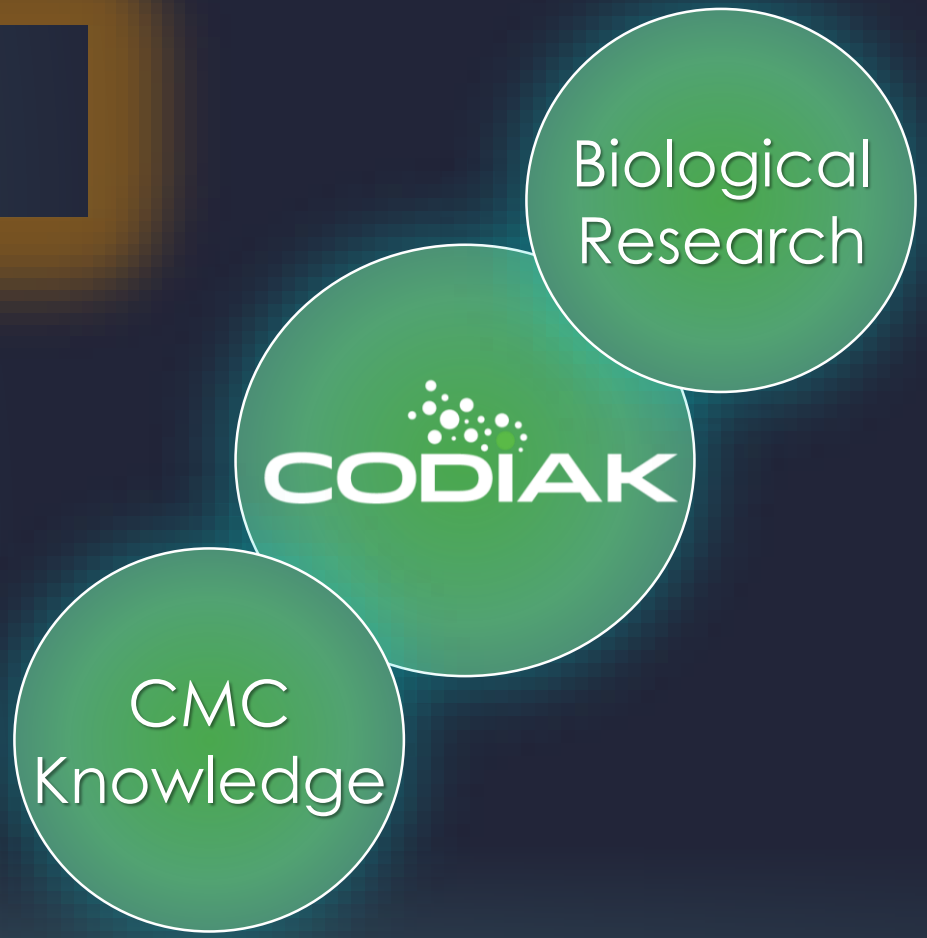


Exosome Science and Bioprocess Engineering have evolved independently



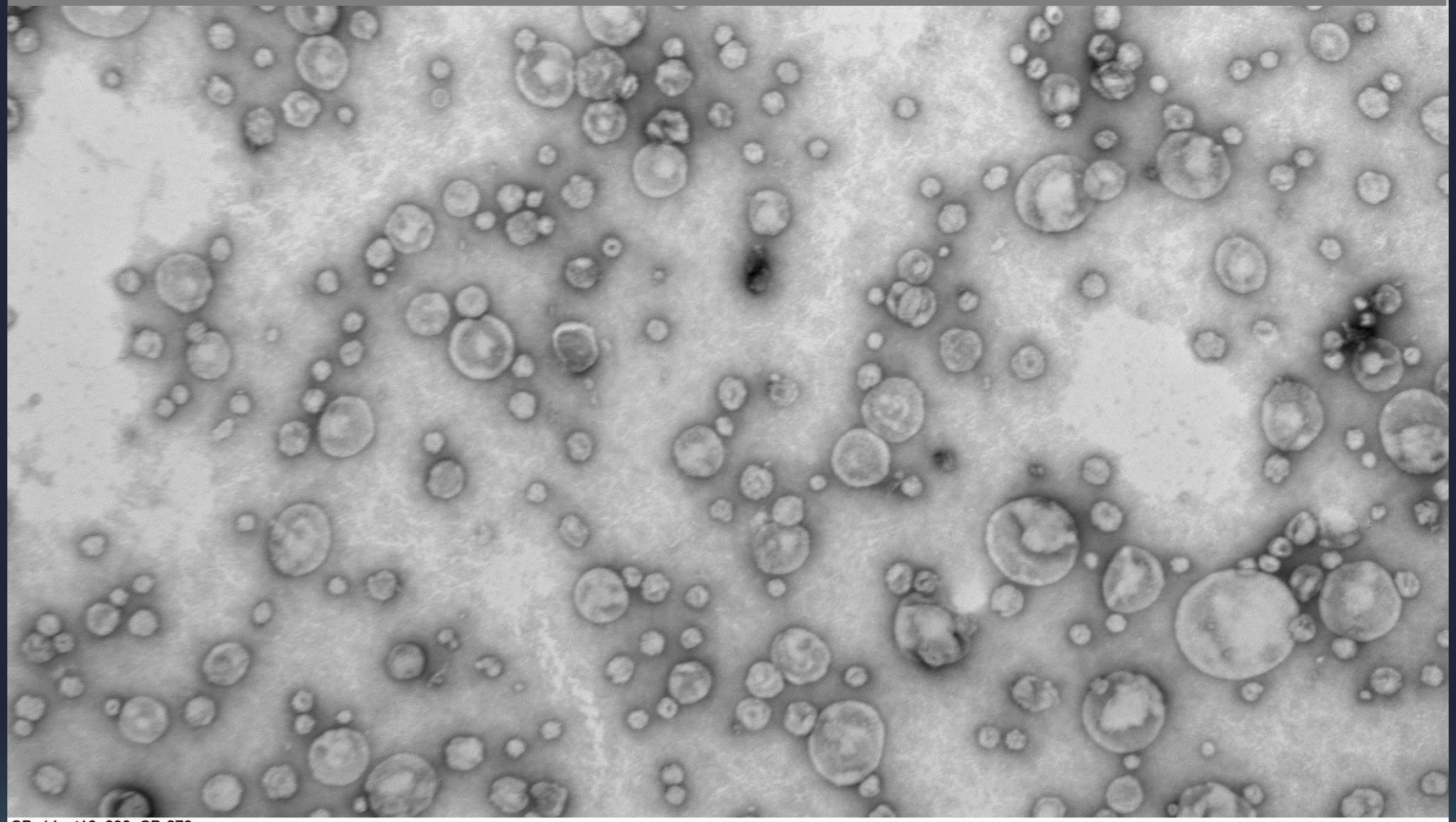
Exosomes: A New Biotherapeutic Modality

- Introduction to Exosome Biology
- Approach to Exosome Manufacturing
- Recent Results and Path Forward



Exosomes: Next Generation Biologicals

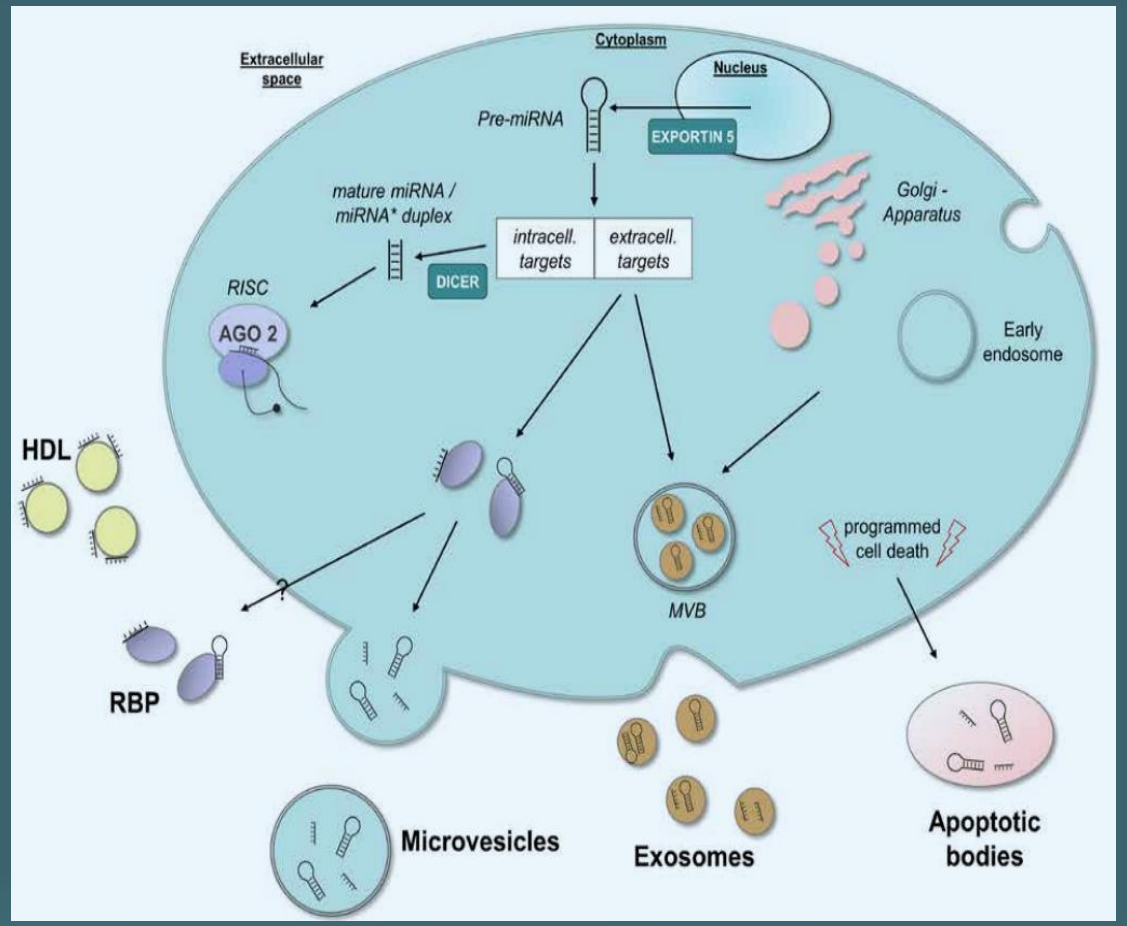
EM image: Purified exosomes from human cells



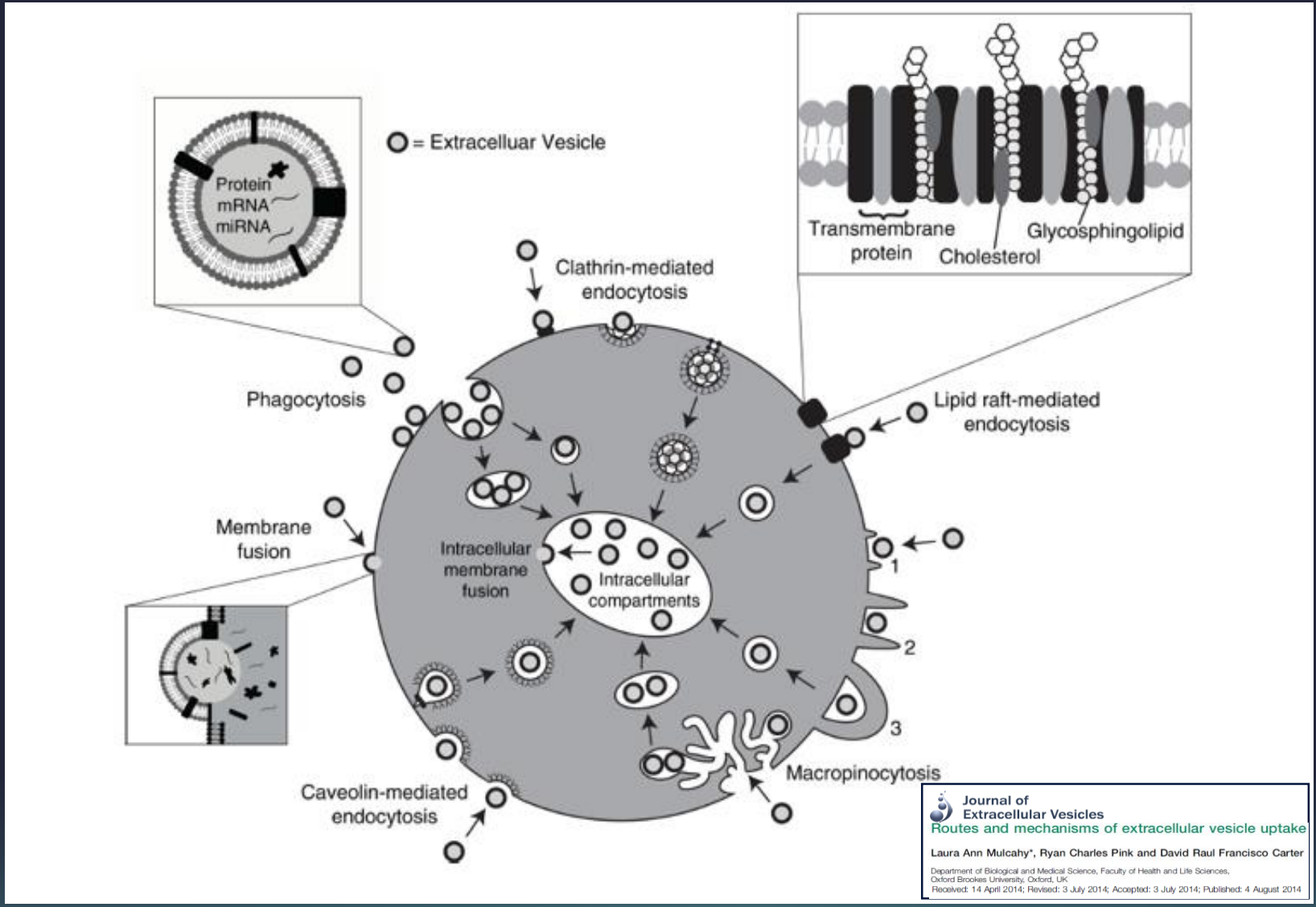
Nikki Ross, Codiak BioSciences exosome isolation data

Exosome Biology

- Subclass of Extracellular Vesicles
- Produced by all living cells
- Native information carrier between tissues
- Protected from the immune system



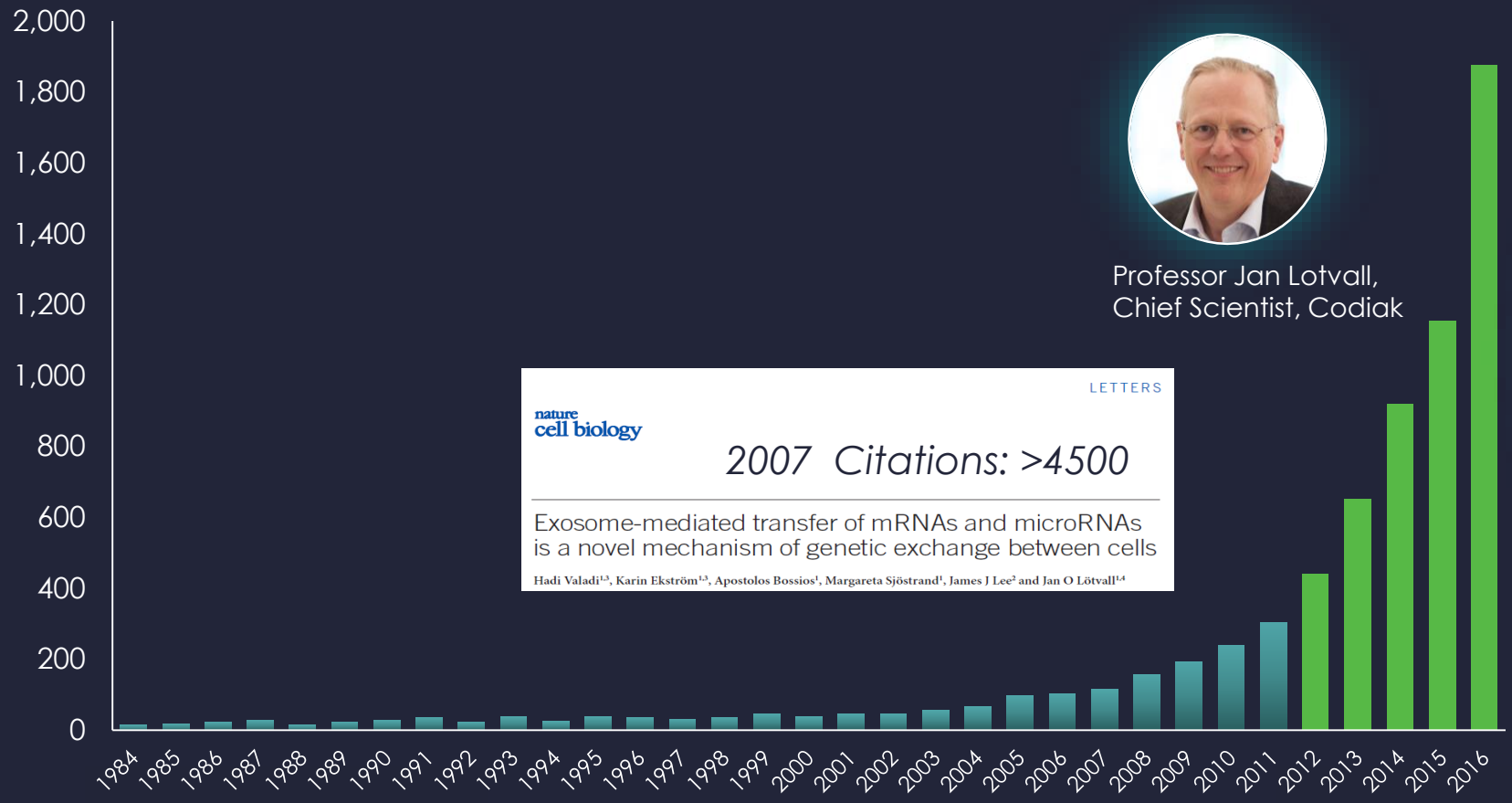
Mechanisms for Exosome Uptake by Cells



Exosome Terminology Can Be Confusing

- Many "-somes" in the literature
  - Exosomes, endosomes, ectosomes, oncosomes, dectosomes, connectosomes, gohstosomes, vexosomes, FedExosomes, …
- No standard definition translatable into characterization methods
- Typical exosome attributes
  - Size (40~200 nm)
  - Canonical surface proteins (CD9, CD47, CD63, CD81, etc.)
  - Relevant functional assays (uptake, biodistribution, etc.)
  - However, above attributes overlap with other Extracellular Vesicles

Explosion in Exosome Knowledge



Professor Jan Lotvall,
Chief Scientist, Codiak

LETTERS

nature cell biology

2007 Citations: >4500

Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells

Hadi Valadi[1,3], Karin Ekström[1,3], Apostolos Bossios[1], Margareta Sjöstrand[1], James J Lee[2] and Jan O Lötvall[1,4]

Since 1983, ~70% Published in Last 5 Years

Peer Reviewed Exosome Publications 1983 – 2016

Rapidly Growing Number of Exosome Companies

CODIAK

Capricor Therapeutics™

Exovita biosciences

evox THERAPEUTICS

ReNeuron

NanoSomix™

exosomedx

Exosome Sciences

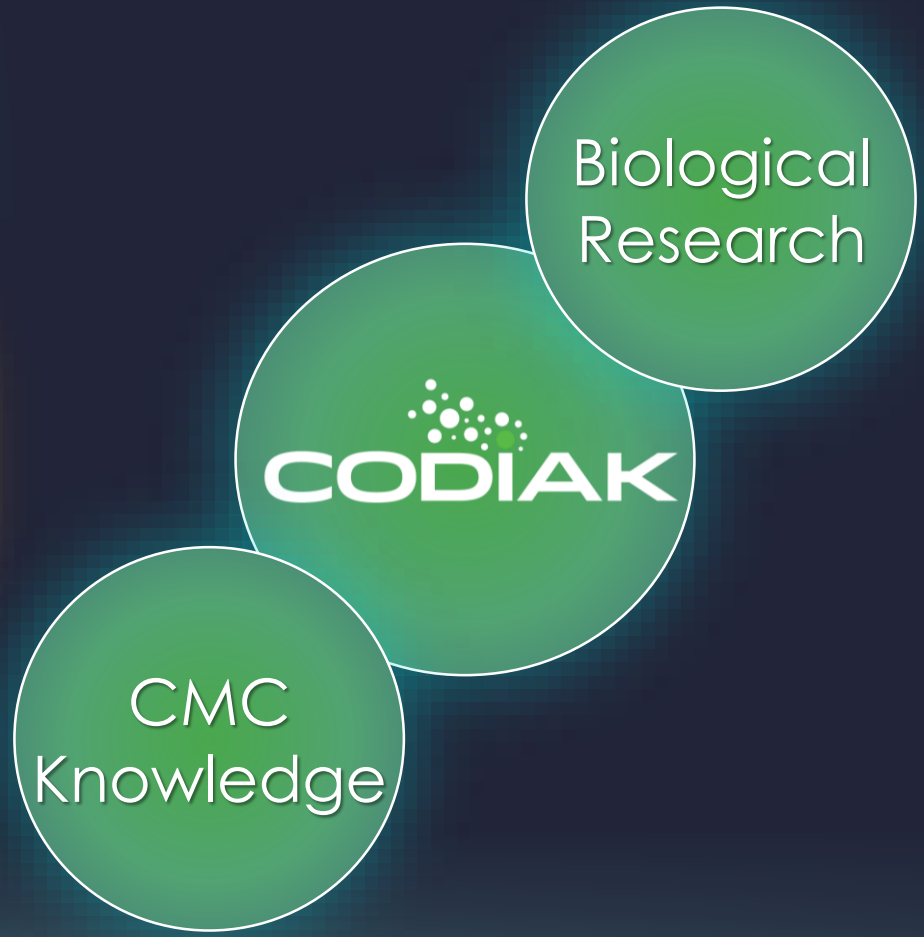
kimera STEM CELL LAB

ExoCoBio

Exosomes for Your Health & Future

Exosomes: A New Biotherapeutic Modality

- Introduction to Exosome Biology
- Approach to Exosome Manufacturing
- Recent Results and Path Forward

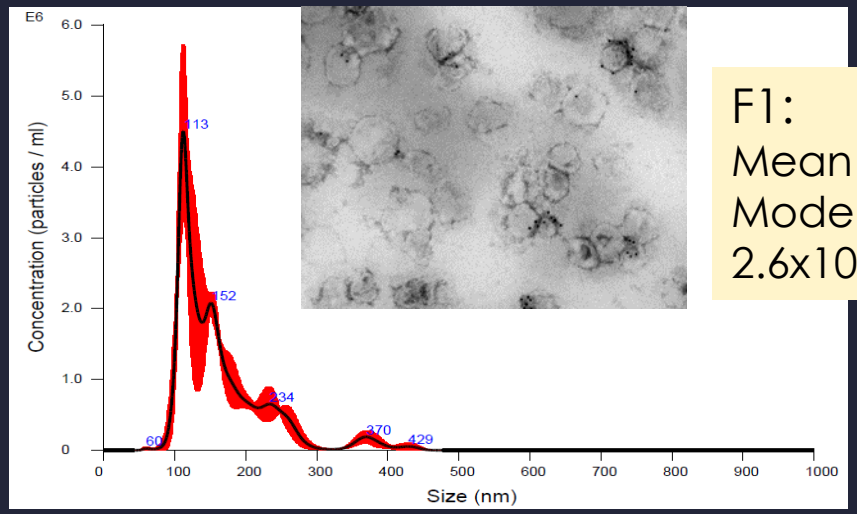


Challenges in Exosome Manufacturing

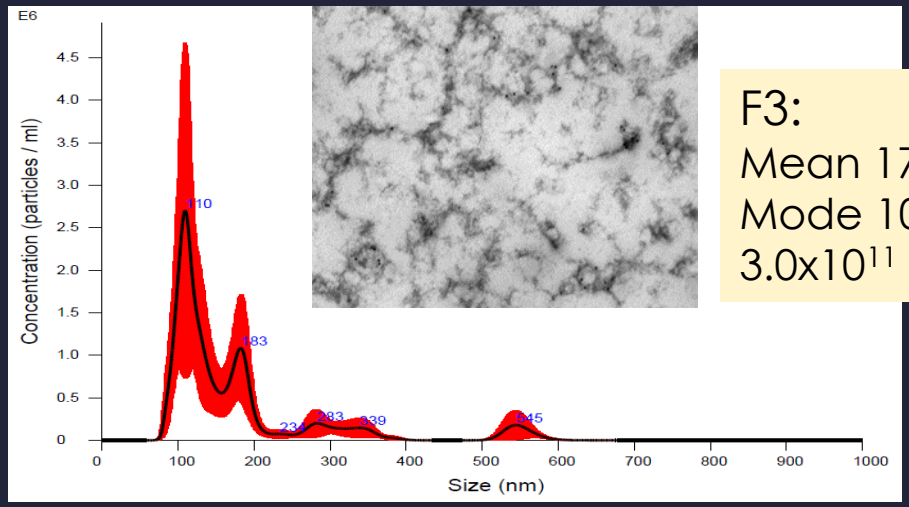
- New scientific field, so far driven mostly by academia. Low level of maturity and standardization
- Limited analytics & characterization
- Insufficient sample purity complicating data interpretation
- High complexity
- Non-scalable exosome production process

Nanoparticle Tracking Analysis (NTA) is used routinely for exosome titer & size characterization

However, NTA does not differentiate exosomes from non-exosome particles

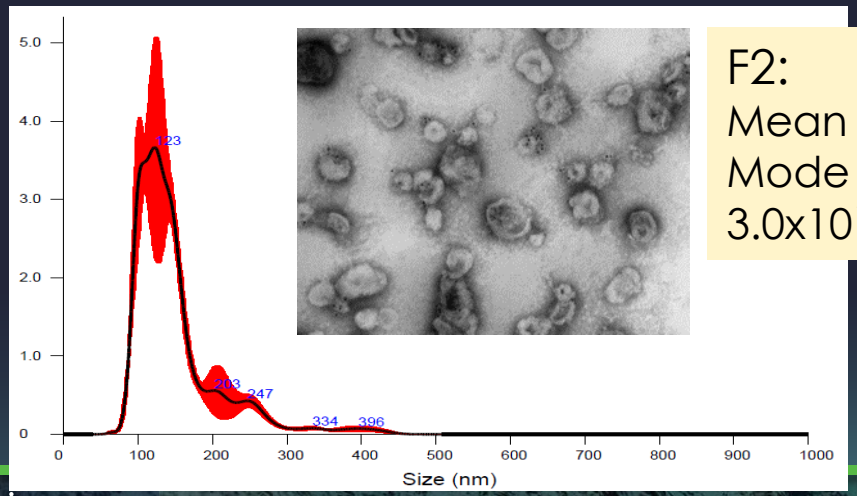


F1:
Mean 162.5nm
Mode 112.1nm
$2.6x10^{11}$ P/ml

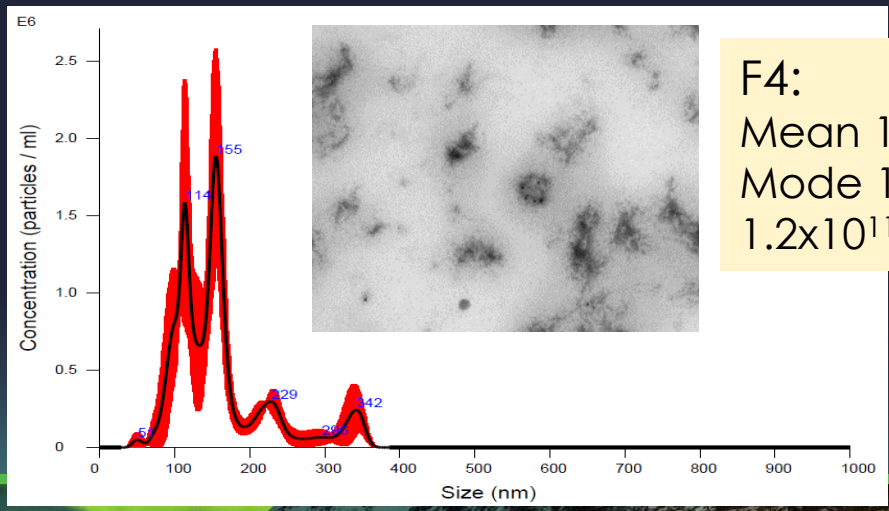


F3:
Mean 173.3nm
Mode 109.4nm
$3.0x10^{11}$ P/ml

F1
F2
F3
F4



F2:
Mean 149.1nm
Mode 122.2nm
$3.0x10^{11}$ P/ml



F4:
Mean 159.1nm
Mode 154.9nm
$1.2x10^{11}$ P/ml

A Typical Current (Academic) Process

Grow adherent cells (typically primary MSCs) in serum

Wash and remove old media, grow cells without serum

2000 g spin, 30 minutes, 4C to remove cells (discard pellet)

12000 g spin, 45 minutes, 4C to remove cell debris (discard pellet)

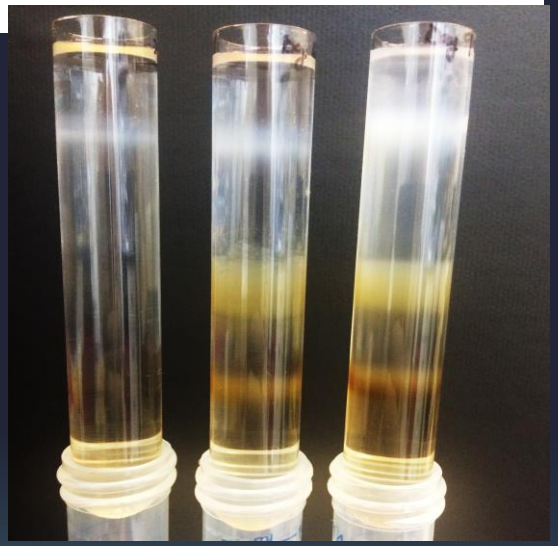
Dilute supernatant in sucrose cushion

110,000 g spin, 120 minutes, 4C (discard supernatant)

Resuspend pellet and wash in PBS

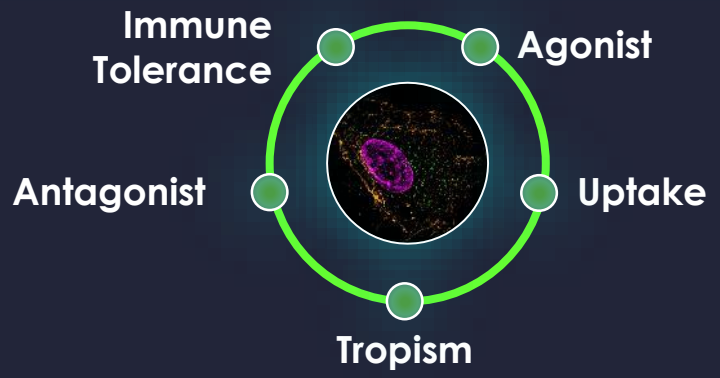
110,000 g spin, 70 minutes, 4C (discard supernatant)

Resuspend pellet and wash in PBS → Perform particle count, freeze in PBS or alternate solution



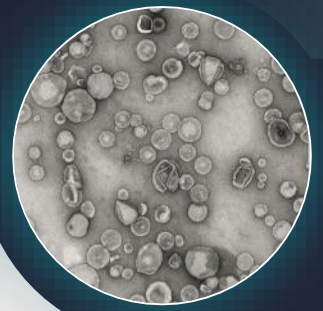
The Codiak Exosome Platform

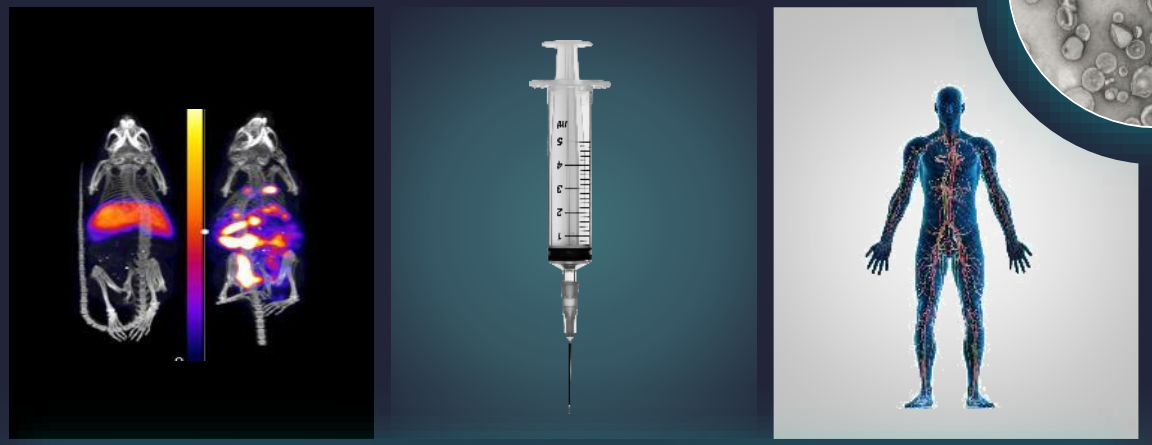
SURFACE ENGINEERING



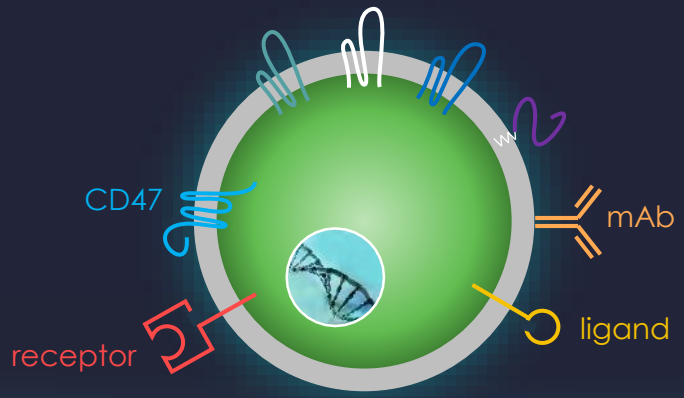
LOADING SPECIFIC PAYLOADS

- Nucleic Acids – DNA, RNA
- Small Molecules
- Antibody
- CRISPR
- Lipids





LOCAL OR SYSTEMIC DELIVERY



COMBINATORIAL POTENTIAL

Plug-and-Play Manufacturing Technology:
Integration of Codiak's Biological and Production Platforms

Three Variable Components (Cell Lines, Payloads, Delivery Routes)

Engineered Cell Lines/Exosomes

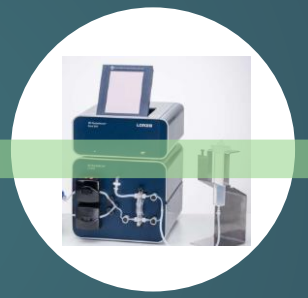
Payloads

Delivery routes

Static Component (Production Process)

Bioreactor

Purification



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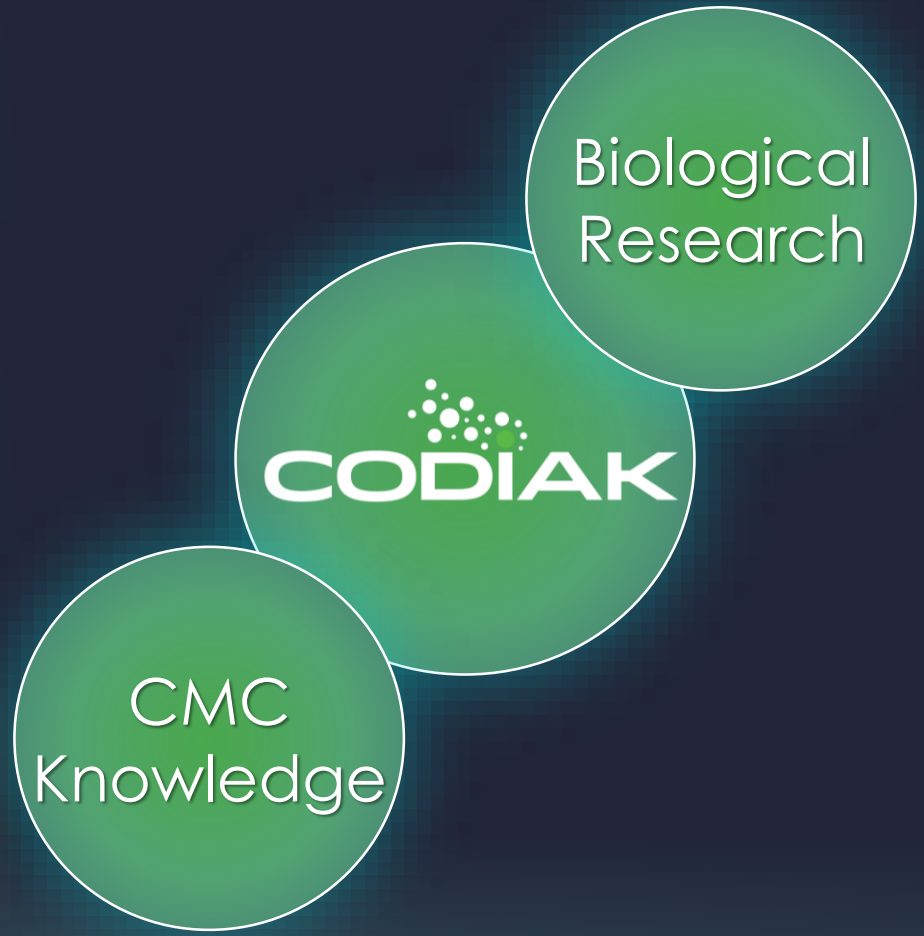
Formulation

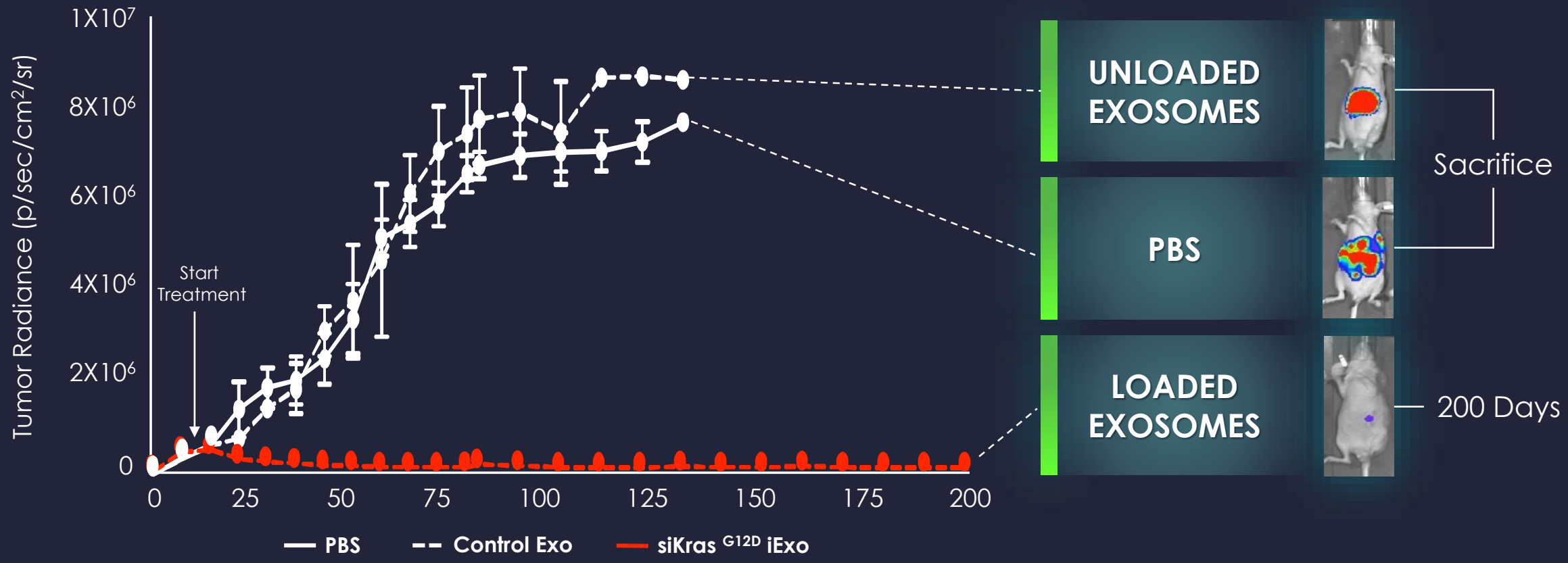
Advanced Upstream, Downstream, Analytics | Scalable | Low COGS

Exosomes: A New Biotherapeutic Modality

- Introduction to Exosome Biology
- Approach to Exosome Manufacturing
- Recent Results and Path Forward



Targeting KRAS with siRNA-Loaded Exosomes



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Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer

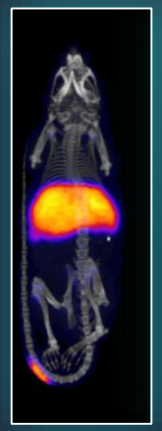
Sushrut Kamerkar, Valerie S. LeBleu, Hikaru Sugimoto, Sujuan Yang, Carolina F. Ruivo, Sonia A., Melo, J. Jack Lee & Raghu Kalluri

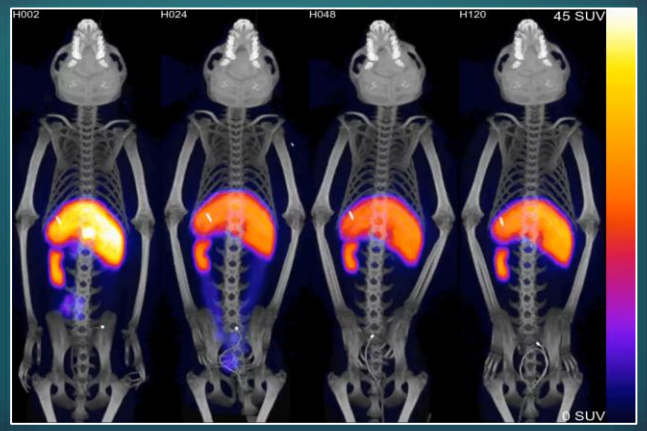
Nature (2017) doi:10.1038/nature22341, Received 20 September 2016 Accepted 03 April 2017 Published online 07 June 2017

Distribution of Exosomes in Healthy Tissues

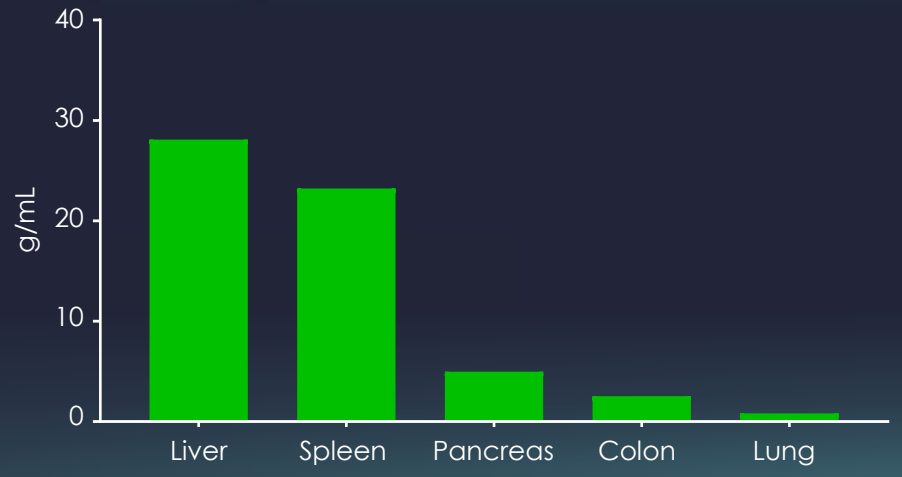
Intra-venous

Mouse



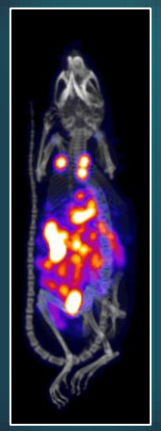


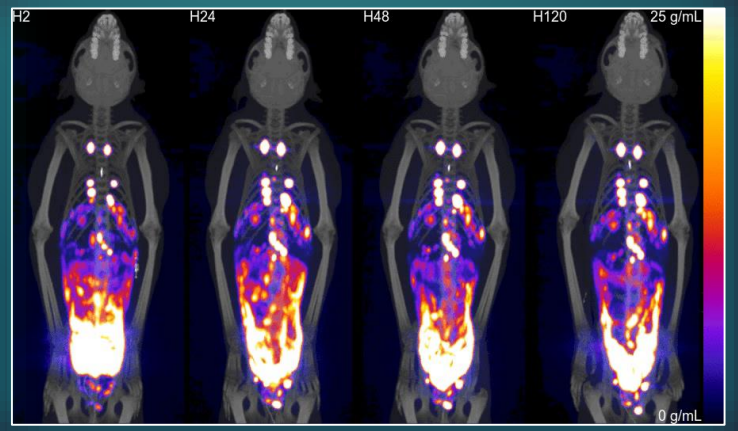
NHP



Intra-peritoneal

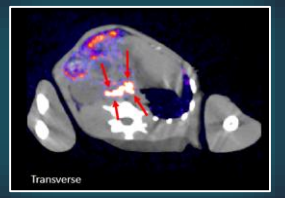
Mouse

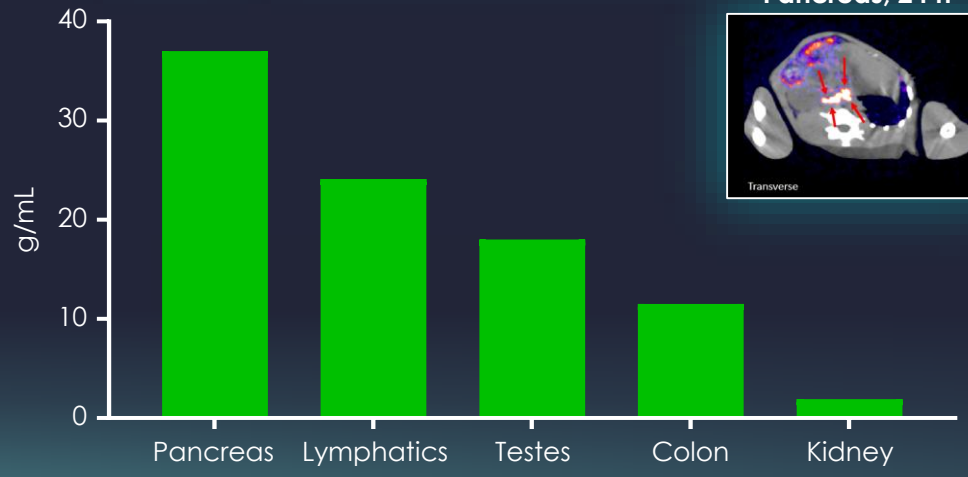




NHP

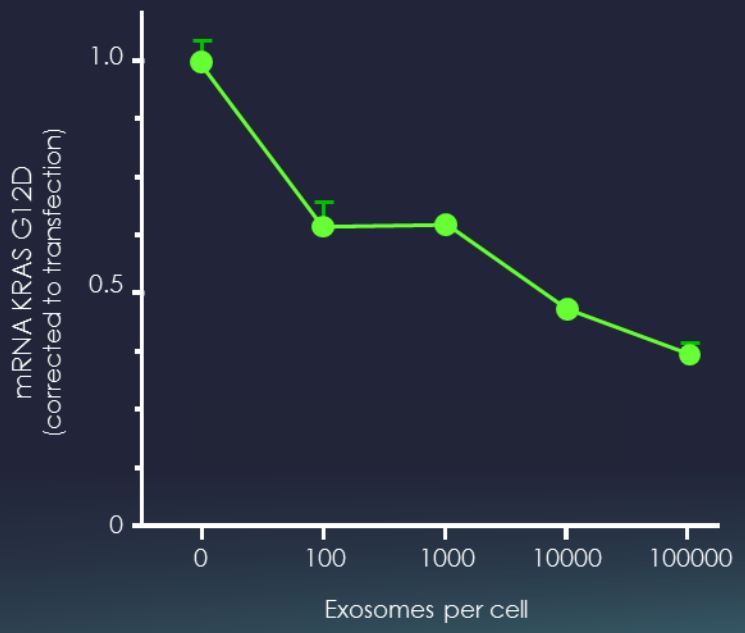
Pancreas, 24 h



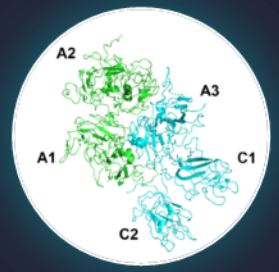


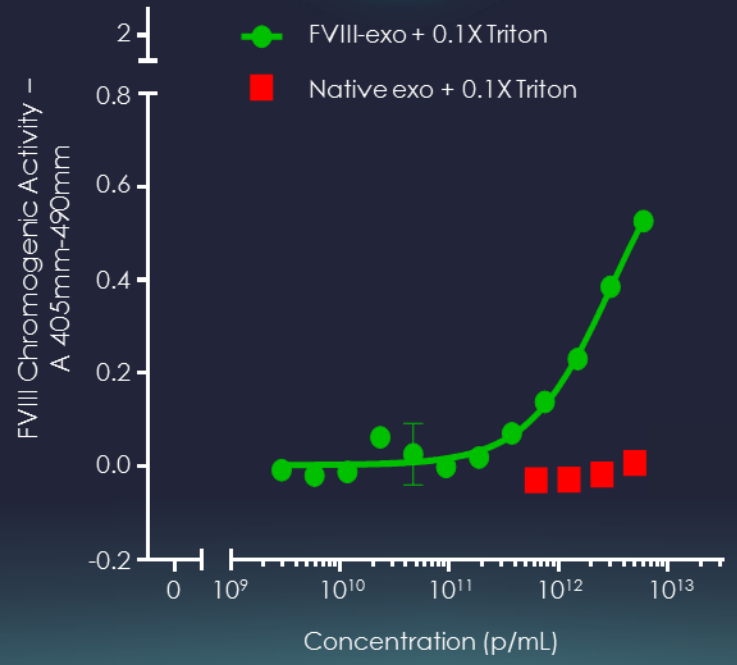
Broad Capacity to Incorporate Cargo

siRNA

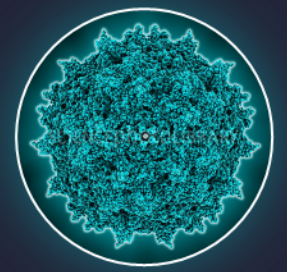


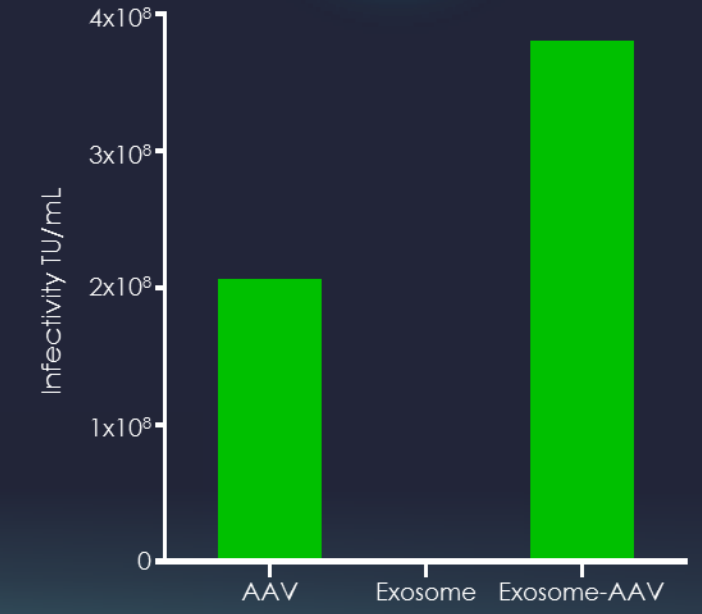
Coagulation Factor VIII





AAV

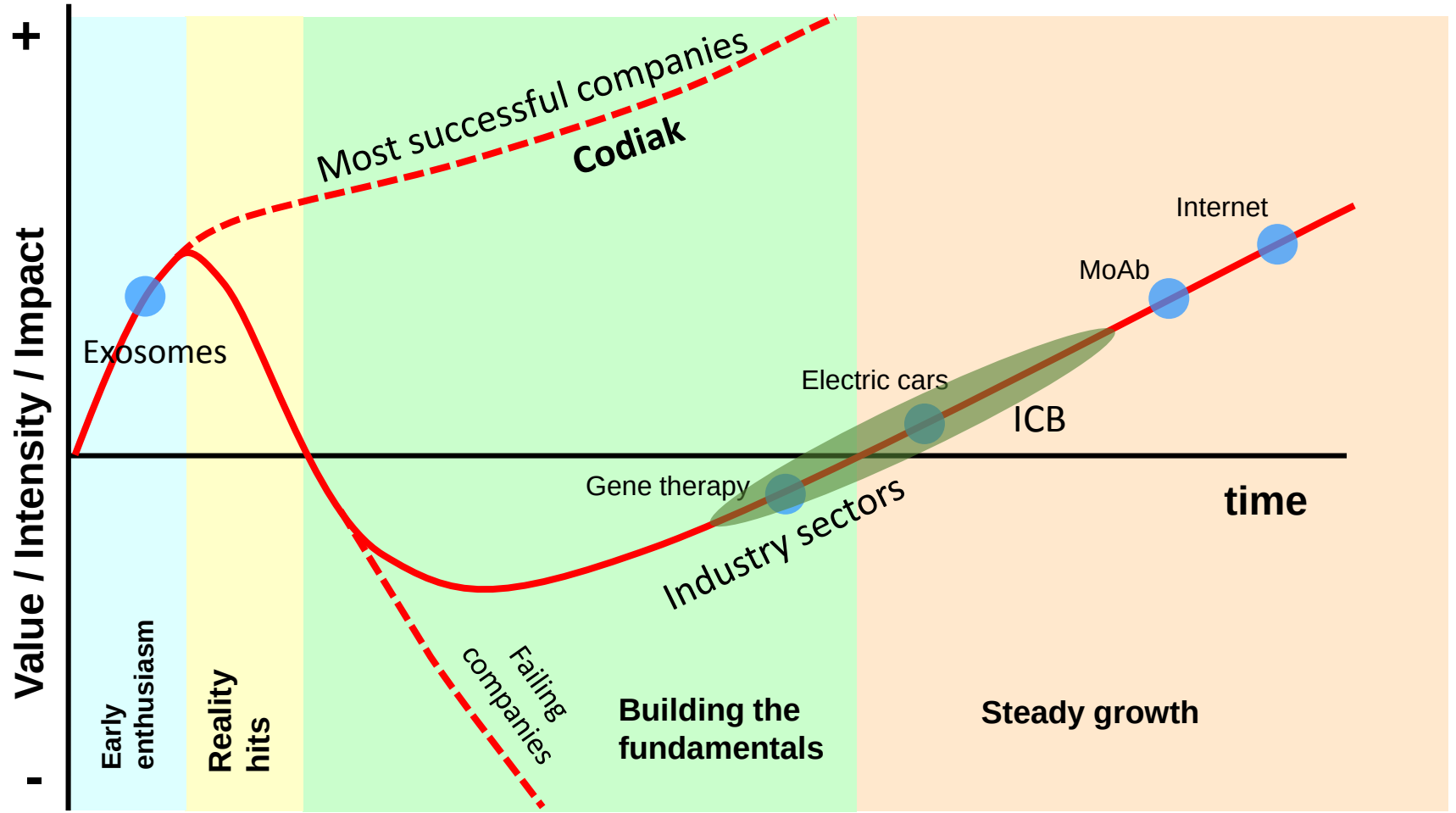




Introduction of Advanced New Technologies

Internet, monoclonal antibody, electric cars, gene therapy, etc.

Where is exosome technology and Codiak?



Moving forward …

Continuous biomanufacturing

+

Exosomes

Stay tuned!

To the awesome ICB community - academia & industry: Thank you !

ICB 3 organizers
- Chetan Goudar
- Suzy Farid
- Paula Alves
- Veena Warikoo

ECI & ICB colleagues & friends
- Manuel Carrondo
- Wei-Show Hu
- Jamie Piret
- Mike Betenbaugh
- Barry Buckland
- Barbara Hickernell
- Tressa D'Ottavio

My Bayer days
- Dr. Hans Henzler
- Chetan Goudar
- Jim Michaels
- Chun Zhang
- John Thrift
- John Murphy
- David Naveh

My Genzyme days
- Veena Warikoo
- Rahul Godawat
- Chris Hwang
- Claudia Buser
- Weichang Zhou
- Jason Walther
- Jin Yin
- Marcella Yu
- Jean McLarty

Codiak
- Scott Estes
- Kathryn Golden
- Agata Villager
- Damian Houde
- Mike Mercaldi

Academia: Prof. Cooney (MIT), Prof. Yoshida (Osaka U)

and many, many others …